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(54) Title: **INTERFERON FOR TREATING OR PREVENTING A CORONAVIRAL INFECTION**

(57) Abstract: The present invention provides a composition and method for use in the prevention or treatment of a coronaviral infection and in particular, the human coronavirus infection termed severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV). A method of treating a coronaviral infection is provided through the administration of interferon, further the use of interferons in the treatment of a coronaviral infection is also provided. Preferred forms of interferon for use in the invention are multi-subtype interferon products such as multi-subtype, human alpha-interferon derived from white blood cells commercially available as Multiferon.

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1       INTERERON FOR TREATING OR PREVENTING A CORONAVIRAL INFECTION

2

3       **Field of the Invention**

4       The present invention provides a composition for use  
5       in the treatment or prevention of a coronavirus  
6       infection, more specifically a human coronaviral  
7       infection, most specifically severe acute  
8       respiratory syndrome (SARS) coronavirus.

9

10      **Background of the Invention**

11      Viral Infection

12      Viral infection is initiated by the binding of a  
13      viral particle to a receptor on the surface of a  
14      host cell membrane. The virus passes into the cell  
15      by endocytosis. Enzymes encoded by the viral genome  
16      are transcribed by the host cell and cause the viral  
17      coat to fuse with the endosome membrane causing the  
18      viral genome to be released into the cytosol. The  
19      virus uses the host cell to effect protein  
20      production in order to make numerous copies of the  
21      genome. Viral coats are formed from coat proteins

1 encoded by the viral genome and synthesised by host  
2 cell ribosomes. The viral genomes are then packaged  
3 into the newly produced viral coats and expelled  
4 from the host cell via the intracellular protein  
5 trafficking pathway or through cell lysis. The  
6 newly synthesised viral particles are then available  
7 for infection of other host cells.

8

9 Coronaviruses

10 Members of the order *Nidovirales*, the coronaviruses  
11 are enveloped, single stranded RNA viruses.  
12 Coronoviral infection causes severe disease of the  
13 respiratory and enteric systems. Coronaviruses have  
14 been associated with gastroenteritis, hepatitis,  
15 peritonitis and bronchitis. However, infection in  
16 humans generally results in milder symptoms. The  
17 SARS human coronavirus (SARS-HCoV) appears to be the  
18 first coronavirus which regularly causes severe  
19 disease in humans. SARS-HCoV causes severe  
20 pneumonia-like symptoms in those infected, with  
21 mortality occurring in the most severe cases.

22

23 Treatment of SARS

24 Various anti-viral treatments have been administered  
25 to humans infected with SARS-HCoV, including general  
26 anti-virals, treatments which inhibit viral cell  
27 entry or replication, and immunostimulants.

28

29 Ribavirin is a broad spectrum anti-viral agent based  
30 on a purine nucleoside analogue and is the standard  
31 treatment regimen for hepatitis C. Ribavirin is  
32 known to be active against various RNA viruses by

1       inducing lethal mutagenesis of the viral RNA genome  
2       (Crotty *et al.*, 2000; Tam *et al.*, 2001) and is known  
3       to show anti-viral activity against animal  
4       coronaviruses (Weiss & Oostrom-Ram, 1989; Sidwell *et*  
5       *al.*, 1987). However, *in vitro* tests of the efficacy  
6       of the drug against SARS-HCoV have produced a series  
7       of negative results and adverse reactions have also  
8       been reported.

9

10      A limited number of other drugs have undergone  
11      testing. The influenza drug, Oseltamivir, a  
12      neuramidase inhibitor, has undergone analysis for  
13      its efficacy against SARS-HCoV infection, but has  
14      not shown any therapeutic benefit (Lee *et al.*, 2003  
15      and Poutanen *et al.*, 2003). In laboratory tests,  
16      Cystatin C, a protease inhibitor found in human  
17      blood, was found to block replication of the 'common  
18      cold' coronaviruses, but this has not been tested  
19      against SARS-HCoV. It is unlikely that Cystatin C  
20      will be a candidate for the treatment of SARS-HCoV  
21      infected patients, since it has not undergone the  
22      safety and efficacy tests required for all human  
23      therapeutics.

24

25      Interferons

26      The interferons (IFNs) may be classified into two  
27      distinct types - Type I IFNs and the Type II IFNs.  
28      The type I IFNs consist of IFN alpha and IFN beta,  
29      whereas the Type II group consists of IFN gamma.  
30      Type I IFNs are produced in direct response to a  
31      viral infection.

32

1 IFN alpha is represented by a large family of  
2 structurally related genes expressing at least  
3 thirteen subtypes, whereas IFN beta is encoded by a  
4 single gene (Diaz et al., 1996). Both types of IFN  
5 are able to stimulate an anti-viral state in target  
6 cells, whereby the replication of a virus is  
7 inhibited through the synthesis of enzymes which  
8 interfere with the cellular and viral processes.

9

10 Type I IFNs also act to inhibit or slow the growth  
11 of target cells and may render them more susceptible  
12 to apoptosis. This has the effect of limiting the  
13 extent of viral spread. Type I IFNs are  
14 immunomodulators, or 'biological response modifiers'  
15 which act to stimulate the immune response. Even  
16 though IFN alpha and IFN beta show many broad  
17 similarities in their actions, there are significant  
18 differences in the manner by which they exert their  
19 effects and it is these extended functions that  
20 account for the different ranges of antiviral  
21 activities of the two types. A review of the  
22 different mechanisms by which interferons exert  
23 their anti-viral effects is provided by Goodbourn et  
24 al., 2000.

25

26 Recombinant interferons, which consist of only the  
27 IFN alpha 2 subtype, currently dominate the market  
28 for anti-viral and oncology indications. The two  
29 main recombinant alpha IFN products, Intron A™ from  
30 Schering Plough (IFN-alpha 2b) and Roferon™ (IFN-  
31 alpha 2a) from Roche. In contrast to these single-  
32 subtype products, there are several alpha IFN

1 preparations that consist of a mixture of different  
2 subtypes. These multi-subtype IFN alpha products  
3 are produced either by human leukocytes in response  
4 to a stimulation from a virus (such as Multiferon™  
5 from Viragen, Inc or its subsidiaries, or Alferon-N™  
6 from Interferon Sciences/Hemispherix), or in human  
7 lymphoblastoid cells, cultured from a patient with  
8 Burkitt's lymphoma (such as Sumiferon™ from  
9 Sumitomo).

10  
11 There are many differences between the recombinant  
12 forms of IFN alpha and the multi-subtype forms. The  
13 most obvious difference is the number of IFN alpha  
14 subtypes each possesses. As mentioned previously,  
15 the recombinant forms comprise only the alpha 2  
16 subtype - the alpha 2b form for Intron A™ (Schering  
17 Plough) and the alpha 2a form for Roferon™ (Roche).  
18 These two allelic variants differ by only one amino  
19 acid residue. The multi-subtype forms of IFN alpha,  
20 as the name suggests, comprise many subtypes of IFN  
21 alpha. Another difference between the multi-subtype  
22 and the recombinant forms is that the IFN alpha 2  
23 produced by human cells in the manufacturing process  
24 of the multi-subtype forms is glycosylated, whereas  
25 the recombinant forms are unglycosylated, in that  
26 they are produced through bacterial fermentation.  
27 Glycosylation plays a major role in many functions  
28 of the protein product, such as half-life, the  
29 bioactivity and its immunogenicity. Therefore, the  
30 glycosylation of a product is an important  
31 consideration when developing a therapeutic or  
32 prophylactic treatment, as it may affect the

1 duration in the body after administration, the  
2 activity of a therapeutically appropriate dose and  
3 the tolerability to the product itself.

4

5 During the last decade, considerable progress has  
6 been achieved in the identification of the  
7 components, as well as the molecular events involved  
8 in the immunotherapeutic effects of interferons.  
9 Over thirty different proteins have been identified  
10 that have been shown to be induced by interferon  
11 (Strannegard, 2002, unpublished review).

12

13 There are currently no completely effective  
14 therapeutic or prophylactic treatments for humans  
15 infected with coronavirus and in particular SARS-  
16 HCoV. There thus exists a need for an effective  
17 treatment for coronaviral infection in humans, and  
18 in particular for severe acute respiratory syndrome  
19 (SARS) coronavirus.

20

21 **Summary of the Invention**

22

23 The present inventors have now shown that  
24 interferons and in particular multiple subtype  
25 natural human alpha interferon products are  
26 surprisingly effective at treating human coronavirus  
27 infection, and in particular severe acute  
28 respiratory syndrome (SARS) coronavirus.

29

30 According to a first aspect of the present invention  
31 there is provided a method of treating coronaviral  
32 infection, the method including the step of

1 administering a therapeutically useful amount of an  
2 interferon to a subject in need of treatment.

3

4 In one preferred embodiment, the method of treatment  
5 can be used to prevent coronaviral infection, the  
6 method including the step of administering a  
7 therapeutically useful amount of an interferon to a  
8 subject sufficient to cause protection against  
9 infection.

10

11 Interferon in each or any of the aspects of the  
12 invention is preferably isolated interferon. An  
13 isolated interferon is an interferon which is  
14 synthetic (e.g. recombinant), or which is altered,  
15 removed or purified from the natural state through  
16 human intervention. For example, an interferon  
17 naturally present in a living animal is not  
18 isolated, whereas a synthetic interferon, or an  
19 interferon which is partially or completely  
20 separated from the coexisting materials of its  
21 natural state, is isolated. An isolated interferon  
22 can exist in substantially purified form, or can  
23 exist in a non-native environment such as, for  
24 example, a cell into which the interferon has been  
25 introduced. Interferons purified from human cells,  
26 for example the multi-subtype, human alpha-  
27 interferon derived from white blood cells  
28 commercially available as Multiferon™ from Viragen,  
29 Inc. or any of its subsidiaries, are also considered  
30 to be isolated molecules for purposes of the present  
31 invention.

32

1     The interferon may be any suitable interferon, for  
2     example interferon alpha or interferon beta. It may  
3     be single or multi-subtype, but is preferably multi-  
4     subtype.

5

6     The interferon may be naturally derived, for example  
7     from human cells or recombinant, but preferably the  
8     interferon is naturally derived. Preferably the  
9     naturally derived interferon is obtained from  
10    leukocytes following viral stimulation or produced  
11    in human lymphoblastoid cells cultured from a  
12    patient with Burkitt's lymphoma.

13

14    Preferred interferons for use in the invention  
15    include multi-subtype interferon alpha (IFN $\alpha$ ),  
16    interferon  $\alpha$ n1, interferon  $\alpha$ n3 or interferon  $\beta$ 1b. A  
17    particularly preferred interferon for use in the  
18    invention is the multi-subtype IFN $\alpha$  product  
19    commercially available from Viragen, Inc. or any of  
20    its subsidiaries under the trade name Multiferon<sup>TM</sup>.

21

22    As used herein the term Multiferon<sup>TM</sup> refers to a  
23    highly purified, multi-subtype, human alpha  
24    interferon derived from human white blood cells  
25    commercially available from Viragen, Inc or any of  
26    its subsidiaries.

27

28    According to a second aspect of the present  
29    invention there is provided an interferon for use in  
30    the treatment or prevention of a coronaviral  
31    infection.

32

1 Preferably the interferon is an isolated interferon.

2

3 The interferon may be any suitable interferon, for  
4 example interferon alpha or interferon beta. It may  
5 be single or multi-subtype, but is preferably multi-  
6 subtype.

7

8 The interferon may be naturally derived, for example  
9 from human cells or recombinant, but preferably the  
10 interferon is naturally derived. Preferably the  
11 naturally derived interferon is obtained from  
12 leukocytes following viral stimulation or produced  
13 in human lymphoblastoid cells cultured from a  
14 patient with Burkitt's lymphoma.

15

16 Preferred interferons for use in the invention  
17 include multi-subtype interferon alpha (IFN $\alpha$ ),  
18 interferon  $\alpha$ n1, interferon  $\alpha$ n3 or interferon  $\beta$ 1b. A  
19 particularly preferred interferon for use in the  
20 invention is the multi-subtype IFN $\alpha$  product  
21 commercially available from Viragen, Inc. or any of  
22 its subsidiaries under the trade name Multiferon<sup>TM</sup>.

23

24 As used herein the term Multiferon<sup>TM</sup> refers to a  
25 highly purified, multi-subtype, human alpha  
26 interferon derived from human white blood cells  
27 commercially available from Viragen, Inc or any of  
28 its subsidiaries.

29

30 According to a third aspect of the present invention  
31 there is provided the use of an interferon in the

1 preparation of a medicament for the treatment or  
2 prevention of a coronaviral infection.

3

4 Preferably the interferon is an isolated interferon.

5

6 The interferon may be any suitable interferon, for  
7 example interferon alpha or interferon beta. It may  
8 be single or multi-subtype, but is preferably multi-  
9 subtype.

10

11 The interferon may be naturally derived, for example  
12 from human cells or recombinant, but preferably the  
13 interferon is naturally derived. Preferably the  
14 naturally derived interferon is obtained from  
15 leukocytes following viral stimulation or produced  
16 in human lymphoblastoid cells cultured from a  
17 patient with Burkitt's lymphoma.

18

19 Preferred interferons for use in the invention  
20 include multi-subtype interferon alpha (IFN $\alpha$ ),  
21 interferon  $\alpha$ n1, interferon  $\alpha$ n3 or interferon  $\beta$ 1b. A  
22 particularly preferred interferon for use in the  
23 invention is the multi-subtype IFN $\alpha$  product  
24 commercially available from Viragen, Inc. or any of  
25 its subsidiaries under the trade name Multiferon<sup>TM</sup>.

26

27 As used herein the term Multiferon<sup>TM</sup> refers to a  
28 highly purified, multi-subtype, human alpha  
29 interferon derived from human white blood cells  
30 commercially available from Viragen, Inc or any of  
31 its subsidiaries.

32

1 Preferably the coronaviral infection is a human  
2 coronaviral infection. Most preferably the  
3 coronaviral infection is severe acute respiratory  
4 system (SARS) coronavirus (SARS-HCoV).

5

6 According to a fourth aspect of the present  
7 invention there is provided a method of treating or  
8 preventing human infection with a coronavirus, and  
9 in particular severe acute respiratory system (SARS)  
10 coronavirus (SARS-HCoV), the method including the  
11 step of administering a therapeutically useful  
12 amount of an interferon to a subject in need of  
13 treatment along with a therapeutically useful amount  
14 of a suitable anti-viral compound.

15

16 In one preferred embodiment, the method of treatment  
17 includes the prevention of human infection with a  
18 coronavirus, wherein the method includes the step of  
19 administering a therapeutically useful amount of an  
20 interferon, or administering an amount of an  
21 interferon along with an amount of a suitable anti-  
22 viral compound sufficient to cause protection  
23 against the infection.

24

25 Preferably the interferon is an isolated interferon.

26

27 Preferably the anti-viral compound is ribavirin.

28

29 Preferably the interferon is any suitable  
30 interferon, for example interferon alpha or  
31 interferon beta. It may be single or multi-subtype,  
32 but is preferably multi-subtype.

1

2 The interferon may be naturally derived, for example  
3 from human cells or of recombinant form, but  
4 preferably the interferon is naturally derived.  
5 Preferably the naturally derived interferon is  
6 obtained from leukocytes following viral stimulation  
7 or produced in human lymphoblastoid cells cultured  
8 from a patient with Burkitt's lymphoma.

9

10 Preferred interferons for use in the invention  
11 include multi-subtype interferon alpha (IFN $\alpha$ ),  
12 interferon  $\alpha$ n1, interferon  $\alpha$ n3 or interferon  $\beta$ 1b. A  
13 particularly preferred interferon for use in the  
14 invention is the multi-subtype IFN $\alpha$  product  
15 commercially available from Viragen, Inc. or any of  
16 its subsidiaries under the trade name Multiferon<sup>TM</sup>.

17

18 As used herein the term Multiferon<sup>TM</sup> refers to a  
19 highly purified, multi-subtype, human alpha  
20 interferon derived from human white blood cells  
21 commercially available from Viragen, Inc or any of  
22 its subsidiaries.

23

24 According to a fifth aspect of the present invention  
25 there is provided the use of interferon and an anti-  
26 viral compound in the preparation of a combined  
27 medicament for the treatment or prevention of  
28 infection with a coronavirus, and in particular  
29 severe acute respiratory system (SARS) coronavirus  
30 (SARS-HCoV).

31

32 Preferably the interferon is an isolated interferon.

1

2 Preferably the anti-viral compound is ribavirin.

3

4 Preferably the interferon is any suitable  
5 interferon, for example interferon alpha or  
6 interferon beta. It may be single or multi-subtype,  
7 but is preferably multi-subtype.

8

9 The interferon may be naturally derived, for example  
10 from humans cell, or of recombinant form, but  
11 preferably the interferon is naturally derived.  
12 Preferably the naturally derived interferon is  
13 obtained from leukocytes following viral stimulation  
14 or produced in human lymphoblastoid cells cultured  
15 from a patient with Burkitt's lymphoma.

16

17 Preferred interferons for use in the invention  
18 include multi-subtype interferon alpha (IFN $\alpha$ ),  
19 interferon  $\alpha$ n1, interferon  $\alpha$ n3 or interferon  $\beta$ 1b. A  
20 particularly preferred interferon for use in the  
21 invention is the multi-subtype IFN $\alpha$  product  
22 commercially available from Viragen, Inc. or any of  
23 its subsidiaries under the trade name Multiferon<sup>TM</sup>.

24

25 As used herein the term Multiferon<sup>TM</sup> refers to a  
26 highly purified, multi-subtype, human alpha  
27 interferon derived from human white blood cells  
28 commercially available from Viragen, Inc or any of  
29 its subsidiaries.

30

31 The term 'treatment' as used herein refers to any  
32 regime that can benefit a human or non-human animal.

1     The treatment may be in respect of an existing  
2     condition or may be prophylactic (preventative  
3     treatment). Treatment may include curative,  
4     alleviation or prophylactic effects.

5

6     Administration

7     Interferons of and for use in the present invention  
8     may be administered alone, or in combination with  
9     another agent, but will preferably be administered  
10    as a pharmaceutical composition, which will  
11    generally comprise a suitable pharmaceutical  
12    excipient, diluent or carrier selected dependent on  
13    the intended route of administration.

14

15    Interferons of and for use in the present invention  
16    may be administered to a patient in need of  
17    treatment via any suitable route. The precise dose  
18    will depend upon a number of factors, including the  
19    precise nature of the interferon.

20

21    Some suitable routes of administration include (but  
22    are not limited to) oral, rectal, nasal, topical  
23    (including buccal and sublingual), vaginal or  
24    parenteral (including subcutaneous, intramuscular,  
25    intravenous, intradermal, intrathecal and epidural)  
26    administration, or administration via oral or nasal  
27    inhalation.

28

29    In preferred embodiments, the composition is  
30    deliverable as an injectable composition, is  
31    administered orally, is administered to the lungs as  
32    an aerosol via oral or nasal inhalation.

1

2 For administration via the oral or nasal inhalation  
3 routes, preferably the active ingredient will be in  
4 a suitable pharmaceutical formulation and may be  
5 delivered using a mechanical form including, but not  
6 restricted to an inhaler or nebuliser device.

7

8 Further, where the oral or nasal inhalation routes  
9 are used, administration by a SPAG (small  
10 particulate aerosol generator) may be used.

11

12 For intravenous injection, the active ingredient  
13 will be in the form of a parenterally acceptable  
14 aqueous solution which is pyrogen-free and has  
15 suitable pH, isotonicity and stability. Those of  
16 relevant skill in the art are well able to prepare  
17 suitable solutions using, for example, isotonic  
18 vehicles such as Sodium Chloride Injection, Ringer's  
19 Injection, Lactated Ringer's Injection.

20 Preservatives, stabilisers, buffers, antioxidants  
21 and/or other additives may be included, as required.

22

23 Pharmaceutical compositions for oral administration  
24 may be in tablet, capsule, powder or liquid form. A  
25 tablet may comprise a solid carrier such as gelatin  
26 or an adjuvant. Liquid pharmaceutical compositions  
27 generally comprise a liquid carrier such as water,  
28 petroleum, animal or vegetable oils, mineral oil or  
29 synthetic oil. Physiological saline solution,  
30 dextrose or other saccharide solution or glycols  
31 such as ethylene glycol, propylene glycol or  
32 polyethylene glycol may be included.

1  
2 The composition may also be administered via  
3 microspheres, liposomes, other microparticulate  
4 delivery systems or sustained release formulations  
5 placed in certain tissues including blood. Suitable  
6 examples of sustained release carriers include  
7 semipermeable polymer matrices in the form of shared  
8 articles, e.g. suppositories or microcapsules.  
9 Implantable or microcapsular sustained release  
10 matrices include polylactides (US Patent No. 3,773,  
11 919 and European Patent Application Publication No  
12 0,058,481) copolymers of L-glutamic acid and gamma  
13 ethyl-L-glutamate (Sidman *et al.*, *Biopolymers* 22(1):  
14 547-556, 1985), poly (2-hydroxyethyl-methacrylate)  
15 or ethylene vinyl acetate (Langer *et al.*, *J. Biomed.*  
16 *Mater. Res.* 15: 167-277, 1981, and Langer, *Chem.*  
17 *Tech.* 12:98-105, 1982, the entire disclosures of  
18 which are herein incorporated by reference).

19  
20 Examples of the techniques and protocols mentioned  
21 above and other techniques and protocols which may  
22 be used in accordance with the invention can be  
23 found in Remington's *Pharmaceutical Sciences*, 16th  
24 edition, Oslo, A. (ed), 1980, the entire disclosure  
25 of which is herein incorporated by reference

26

27 Pharmaceutical Compositions

28 As described above, the present invention extends to  
29 a pharmaceutical composition for the treatment or  
30 prevention of a coronaviral infection, wherein the  
31 composition comprises at least one interferon.

32 Pharmaceutical compositions according to the present

1 invention, and for use in accordance with the  
2 present invention may comprise, in addition to  
3 active ingredient (i.e. one or more interferons), a  
4 pharmaceutically acceptable excipient, carrier,  
5 buffer stabiliser or other materials well known to  
6 those skilled in the art. Such materials should be  
7 non-toxic and should not interfere with the efficacy  
8 of the active ingredient. The precise nature of the  
9 carrier or other material will depend on the route  
10 of administration, which may be, for example, oral,  
11 intravenous, or intranasal.

12

13 The formulation may be a liquid, for example, a  
14 physiologic salt solution containing non-phosphate  
15 buffer at pH 6.8 to 7.6, or a lyophilised powder.

16

17 Dose

18 The composition/interferon is preferably  
19 administered to an individual in a "therapeutically  
20 effective amount", this being sufficient to show  
21 benefit to the individual. The actual amount  
22 administered, and rate and time-course of  
23 administration, will depend on the nature and  
24 severity of what is being treated. Prescription of  
25 treatment, e.g. decisions on dosage etc, is  
26 ultimately within the responsibility and at the  
27 discretion of general practitioners and other  
28 medical doctors, and typically takes account of the  
29 disorder to be treated, the condition of the  
30 individual patient, the site of delivery, the method  
31 of administration and other factors known to  
32 practitioners.

1  
2     The optimal dose can be determined by physicians  
3     based on a number of parameters including, for  
4     example, age, sex, weight, severity of the condition  
5     being treated, the active ingredient being  
6     administered and the route of administration.

7

8     For example, in one embodiment, a suitable dose of  
9     interferon may be 1 to 10 million IU, for example 3  
10    to 5 million IU three times weekly to 0.5 to 10  
11    million, for example 2 to 8 million, or 4 to 6  
12    million IU daily, although other doses may be used.

13

14    According to a further aspect of the present  
15    invention there is provided an assay method for  
16    determining the efficacy of a composition in the  
17    treatment or prevention of a coronaviral infection,  
18    wherein the composition comprises an interferon,  
19    preferably a multi sub-type interferon.

20

21    In a further aspect of the present invention, there  
22    is provided an assay method for determining the  
23    efficacy of a candidate agent in the treatment of a  
24    coronaviral infection, wherein the assay method  
25    includes the steps of;

26        - incubating virus infected cells in the  
27        presence of the candidate agent, and  
28        - determining the degree of inhibition of the  
29        cytopathic effect of the virus on the cells.

30

31

1 Preferably the method includes the further step of  
2 comparing the degree of viral inhibition obtained  
3 using the candidate agent with the degree of viral  
4 inhibition obtainable with incubation with an  
5 interferon or interferon based product.

6

7 Preferably the interferon is a multi-subtype  
8 interferon, most preferably Multiferon™.

9

10 In a still further aspect, there is provided an  
11 assay method for determining the efficacy of a  
12 candidate agent in the prevention of a coronaviral  
13 infection, wherein the assay method includes the  
14 steps of:

15 -incubating cells in the presence of the candidate  
16 agent,  
17 -adding the coronavirus to the cells, and  
18 -determining the degree of protection against the  
19 coronaviral infection afforded by the candidate  
20 agent

21

22 Preferred assays for use in the assay methods of the  
23 invention include cytopathic endpoint assays and  
24 plaque reduction assays.

25

26 Preferred features of each aspect of the invention  
27 are as for each of the other aspects *mutatis*  
28 *mutandis* unless the context demands otherwise.

29

30 Unless otherwise defined, all technical and  
31 scientific terms used herein have the meaning

1 commonly understood by a person who is skilled in  
2 the art in the field of the present invention.

3

4 Throughout the specification, unless the context  
5 demands otherwise, the terms 'comprise' or  
6 'include', or variations such as 'comprises' or  
7 'comprising', 'includes' or 'including' will be  
8 understood to imply the inclusion of a stated  
9 integer or group of integers, but not the exclusion  
10 of any other integer or group of integers.

11

12 **Detailed description of the Invention**

13

14 The present invention will now be described with  
15 reference to the following examples which are  
16 provided for the purpose of illustration and are not  
17 intended to be construed as being limiting on the  
18 present invention, and further, with reference to  
19 the figures.

20

21 **Brief description of the drawings**

22

23 Figure 1 shows a dose response curve produced  
24 from an *in vitro* plaque reduction assay,  
25 showing that with increasing concentrations of  
26 the Multiferon™, the effect of the SARS-HCoV  
27 virus is attenuated;

28

29 Figure 2 shows the effect of Multiferon™ and  
30 Intron A™ on the cytopathogenicity of Semliki  
31 Forest Virus (SFV) on African Green Monkey  
32 Kidney Vero E6 cells;

1

2       Figure 3 shows the effect of Multiferon™ on the  
3       cytopathogenicity of human Encephalomyocarditis  
4       virus (EMCV) on human A459 cells, wherein the  
5       Multiferon™ concentration required to obtain  
6       50% cytopathic effect (CPE) for human A459  
7       cells challenged with EMC virus is shown for  
8       different concentrations of EMC virus,  
9       presented as a 1/dilution; and

10

11       Figure 4 shows the effect of increasing  
12       concentrations of Multiferon™ on human A459  
13       cell survival. Cell survival was measured  
14       photometrically at Abs<sub>595nm</sub> using a fixed  
15       dilution of EMC virus (dilution 1/400), at  
16       increasing concentrations of Multiferon. AU  
17       denoted Absorbance Units.

18

19       **Examples**

20

21       Example 1 - Anti-viral effect of interferon against  
22       SARS-HCoV infection in Vero E6 cells

23

24       The effectiveness of the interferons to inhibit the  
25       cytopathic effect following SARS-HCoV infection was  
26       tested in a cytopathic endpoint assay and a plaque  
27       reduction assay. All endpoint assays were carried  
28       out using the multi-subtype interferons Multiferon™  
29       and interferon  $\alpha$ n3, as well as single subtype  
30       recombinant interferon alpha (subtypes interferon  
31        $\alpha$ 2a, interferon  $\alpha$ 2b, and interferon  $\alpha$ n1) and the  
32       interferon beta (IFN $\beta$ ) subtypes interferon  $\beta$ 1a and

1 interferon  $\beta$ 1b as well as the anti-viral Ribavirin  
2 for comparison.

3

4 Preparation of anti-viral treatments

5 A broad range of concentrations (obtained by ten-  
6 fold dilutions) encompassing the inhibitory dosages  
7 stated by the manufacturer for other viral-host  
8 combinations was tested. Compounds already present  
9 in aqueous injections were made up to volume using  
10 Hank's buffered saline solution. For tablet and  
11 capsule formulations with soluble active  
12 ingredients, the outer coat was removed wherever  
13 applicable and the preparation ground in a mortar  
14 and pestle. The contents were dissolved in water,  
15 vortexed and centrifuged thereafter at 3000G. The  
16 required volume was pipetted from the supernatant  
17 and diluted accordingly. Where active ingredients  
18 were insoluble in water, the contents were dissolved  
19 in dimethylsulphoxide (DMSO) and care was taken to  
20 ensure that the final concentration of DMSO in the  
21 dilutions would not exceed 1%. For plaque assays,  
22 5-fold drug dilutions were prepared using growth  
23 media as specified below.

24

25 SARS-HCoV production and infection

26 African Green Monkey (Vero E6) cells (American Type  
27 Culture Collection, Manassas, VA, USA) were  
28 propagated in 75cm<sup>2</sup> cell culture flasks containing  
29 growth medium consisting of medium 199 (Sigma, St  
30 Louis, USA) supplemented with 10% foetal calf serum  
31 (FCS; Biological Industries, Israel). SARS-HCoV  
32 2003VA2774 (an isolate from a SARS patient in

1 Singapore) was propagated in Vero E6 cells.  
2 Briefly, 2 ml of stock virus was added to a  
3 confluent monolayer of Vero E6 cells and incubated  
4 at 37°C in 5% CO<sub>2</sub> for one hour. 13 ml of medium 199  
5 supplemented with 5% FCS was then added. The  
6 cultures were incubated at 37°C in 5% CO<sub>2</sub> and the  
7 inhibition of cytopathic effect gauged by observing  
8 each well through an inverted microscope. Where 75%  
9 or greater inhibition was observed after 48 hours,  
10 the supernatant was harvested. The supernatant was  
11 clarified at 2500 rpm and then aliquoted into  
12 cryovials and stored at -80°C until use.  
13

14 Virus handling and titration

15 Virus titre in the frozen culture supernatant was  
16 determined using a plaque assay carried out in  
17 duplicate. Briefly, 100 microlitres of virus in 10-  
18 fold serial dilution was added to a monolayer of  
19 Vero E6 cells in a 24 well-plate. After incubation  
20 for an hour at 37°C in 5% CO<sub>2</sub>, the viral inoculum was  
21 aspirated and 1 ml of carboxymethylcellulose overlay  
22 with medium 199 supplemented with 5% FCS was added  
23 to each well. After four days of incubation, the  
24 cells were fixed with 10% formalin and stained with  
25 2% crystal violet. The plaques were counted  
26 visually and the virus titre in plaque forming units  
27 per ml (pfu/ml) calculated.  
28

29 Cytopathic endpoint assay

30 The protocol used was adapted from Al-Jabri *et al.*  
31 1996. The effect of each anti-viral treatment was  
32 tested in quadruplicate. Briefly, 100 microlitres

1 of serial 10-fold dilutions of each treatment were  
2 incubated with 100 microlitres of Vero E6 cells  
3 giving a final cell count of 20,000 cells per well  
4 in a 96-well plate. Incubation was at 37°C in 5% CO<sub>2</sub>  
5 overnight for the interferon preparations and for  
6 one hour for Ribavirin. 10 microlitres of virus at  
7 a concentration of 10,000 pfu/well were then added  
8 to each test well. This equates to a multiplicity  
9 of infection (MOI) (virus particles per cell) of 0.5.  
10 The plates were incubated at 37°C in 5% CO<sub>2</sub> for three  
11 days and the plates were observed daily for  
12 cytopathic effects. The end point was the diluted  
13 concentration that inhibited the cytopathic effect  
14 in all four set-ups (CIA<sub>100</sub>).

15

16 To determine cytotoxicity, 100 microlitres of serial  
17 10-fold dilutions of each treatment were incubated  
18 with 100 microlitres of Vero E6 cells giving a final  
19 cell count of 20,000 cells per well in a 96-well  
20 plate, without viral challenge. The plates were  
21 then incubated at 37°C in 5% CO<sub>2</sub> for three days and  
22 toxicity effects were observed for using an inverted  
23 microscope.

24

25 Interferons which showed complete inhibition were  
26 tested further at the lower viral titres of 10<sup>3</sup> and  
27 10<sup>2</sup> pfu/well.

28

29 Plaque reduction assay

30 Multiferon<sup>TM</sup>, interferon  $\alpha$ n3 and interferon  $\beta$ 1b were  
31 further tested using a plaque reduction assay.  
32 Trypsinised Vero E6 cells were re-suspended in

1      growth medium and pre-incubated for 15 hours with a  
2      serial 5-fold dilution of interferon  $\alpha$ n3, interferon  
3       $\beta$ 1a and Multiferon<sup>TM</sup> in 24-well plates. The following  
4      day, the medium was aspirated and 100 microlitres of  
5      virus was added to each well at a titre of 100  
6      pfu/well.

7

8      After incubation for one hour, the virus inoculum  
9      was aspirated and a carboxymethylcellulose overlay  
10     containing maintenance medium and the appropriate  
11     interferon concentration was added. After four days  
12     incubation, the plates were fixed and stained as  
13     described above.

14

15     Viral plaques were visible 3 days after pre-  
16     incubation of infected cells for 15 hours with five-  
17     fold dilutions of the interferon. Plaques were then  
18     counted visually and the concentration of the  
19     interferon which inhibited 50% of plaques in each  
20     well ( $IC_{50}$ ) determined. Results were plotted in  
21     Microsoft Excel, and a polynomial of order three was  
22     used to approximate the data and extrapolate  $IC_{50}$  and  
23      $IC_{95}$  values. (Results not shown)

24

25     The assay was also carried out in duplicate as  
26     described above for Multiferon<sup>TM</sup> at a viral titre of  
27     54 pfu/well.

28

29     Interferons are known to be relatively species  
30     specific as the target for the interferon is the  
31     infected cell rather than the virus itself. The  
32     anti-viral activity of Multiferon<sup>TM</sup> was also assessed

1 in a human cell line, the pulmonary epithelial cell  
2 line A549.

3

4 Results

5

6 Cytopathic Endpoint Assay

7 The cytopathic effect of SARS-HCoV was evident  
8 within 24 hours following infection. Infected cells  
9 were rounded and exhibited monolayer destruction.

10

11 Complete inhibition using a high viral challenge  
12 ( $10^4$  pfu/well) and high multiplicity of infection  
13 (0.5) was observed for Ribavirin<sup>TM</sup>, and for the  
14 Multiferon<sup>TM</sup> product. At a viral load of  $10^2$   
15 pfu/well the CIA<sub>100</sub> value was 5 IU/ml for  
16 Multiferon<sup>TM</sup>, with no cytotoxicity observed.

17

18 Although Ribavirin<sup>TM</sup> showed inhibitory activity at  
19 all viral titres this was only at high  
20 concentrations of the drug. Such concentrations  
21 showed cytotoxicity and thus Ribavirin<sup>TM</sup> is not  
22 likely to be a clinically effective treatment for  
23 severe acute respiratory syndrome (SARS)  
24 coronavirus.

25

26 In contrast, Multiferon<sup>TM</sup> did not show any  
27 cytotoxicity at this inhibitory concentration.

28

29 Interferon  $\alpha$ n3, interferon  $\alpha$ n1 and interferon  $\beta$ 1b  
30 also showed inhibition of cytopathic effect using  
31 this assay. Interferon  $\alpha$ 2a, interferon  $\alpha$ 2b and

1 interferon  $\beta$ 1a did not show significant inhibition  
 2 (results not shown).

3

4 Results are shown for Multiferon<sup>TM</sup> and Ribavirin<sup>TM</sup> in  
 5 Tables 1 and 2 below.

6

Anti-viral Treatment	Concentration at which complete cytopathic effect	CIA <sub>100</sub>
Multiferon <sup>TM</sup>	5,000 IU/ml	Yes
Ribavirin <sup>TM</sup>	5,000 $\mu$ g/ml	Yes

7 **Table 1:** Results of the Cytopathic Endpoint Assay  
 8 for Multiferon<sup>TM</sup> and Ribavirin<sup>TM</sup>. (Results not shown  
 9 for other treatments tested)

10

Virus Load (pfu/well)	Multiferon <sup>TM</sup> (IU/ml)	Ribavirin <sup>TM</sup> ( $\mu$ g/ml)
1,000	50	5,000
100	5	500

11 **Table 2:** Data obtained for Multiferon<sup>TM</sup> and the anti-  
 12 viral product, Ribavirin<sup>TM</sup>. (Results not shown for  
 13 the other treatments tested).

14

15 Plaque Reduction Assay

16 The Multiferon<sup>TM</sup> preparation displayed a dose-  
 17 dependent inhibition of SARS-HCoV plaque formation.  
 18 IC<sub>50</sub> and IC<sub>95</sub> values for Multiferon<sup>TM</sup> treatment were 2  
 19 IU/ml and 44 IU/ml, respectively. Results are shown  
 20 below for Multiferon<sup>TM</sup> in Table 3 and in Figure 1 for  
 21 a viral titre of 54 pfu/well. An EC<sub>50</sub> value of 3.16  
 22 IU/ml was obtained.

1

Multiferon™ Concentration (IU/ml)	Log Multiferon™ Concentration (Log IU/ml)	% plaque reduction (Well 1)	% plaque reduction (Well 2)	Average plaque reduction
5000	3.69897	100	100	100
1000	3	100	100	100
200	2.30103	100	100	100
40	1.60206	100	100	100
8	0.90309	68.5	75.9	72.2
1.6	0.20412	40.7	48.1	44.4
0.32	-0.49485	18.5	25.9	22.2
0.064	-1.19382	0	0	0

2      **Table 3:** Results obtained in the plaque reduction  
 3      assay for Multiferon™ at 54 pfu/well.

4

5      Interferon  $\alpha$ n3 and interferon  $\beta$ 1a also showed dose-  
 6      dependent inhibition of SARS-HCoV plaque formation  
 7      in this assay (results not shown).

8

9      Example 2

10

11      SARS-HCoV, strain Frankfurt-1, kindly provided by  
 12      the Bernard Notch Institute, Frankfurt, Germany, was  
 13      propagated on Vero E6 cells, an African Green Monkey  
 14      cell line obtained from American Type Culture  
 15      Collection, Manassas, VA, USA. For titration of the  
 16      virus, serial dilution of SARS-HCoV were added to  
 17      Vero E6 cells grown in micro-plates with Eagle's  
 18      medium containing 2% foetal calf serum. After 3  
 19      days of culture, cytopathogenic effects were  
 20      determined microscopically and cytotoxicity was then  
 21      assayed using a colorimetric assay based on the  
 22      measurement of lactate dehydrogenase (LDH) activity

1 released from the cytosol of damaged cells  
2 (Cytotoxicity detection kit, Roche Diagnostics GmbH,  
3 Penzberg, Germany).

4

5 For the antiviral experiments the following four  
6 different commercially available interferon  
7 preparations were used: 1) Intron A<sup>TM</sup>, Schering  
8 Plough, USA; 2) Roferon<sup>TM</sup>, Roche, Switzerland; 3)  
9 Betaferon<sup>TM</sup>, Schering AG, Germany and 4) Multiferon<sup>TM</sup>  
10 (Viragen, Florida, USA).

11

12 Serial 5-fold dilutions (0.2-31.125 IU/ml) of the  
13 interferon preparations were added to Vero E6 cells  
14 in micro-plates which were then incubated overnight  
15 at 37°C. SARS-HCoV was then added at different  
16 concentrations (1000, 100 or 10 TCID<sub>50</sub>) to different  
17 sets of interferon dilutions, and after a further  
18 incubation of 3 days the plates were read  
19 microscopically, and then by the ELISA LDH  
20 cytotoxicity assay.

21

22 In a separate set of experiments, the method used by  
23 Cinatl *et al.* (2003) including addition of  
24 interferon on two occasions, one day before and one  
25 day after addition of the virus to the plates, was  
26 employed.

27

28 In all experiments, controls with 1) virus but not  
29 interferon, 2) all different dilutions of the  
30 interferons but no virus, and 3) no virus and no  
31 interferon were included.

32

1      Results

2      The cytotoxicity (LDH) assay used for determination  
3      of SARS-HCoV cytopathogenic effect (CPE) was found  
4      to be highly reliable, giving OD values in CPE-  
5      positive cultures of 1.5-1.8 and in CPE-negative  
6      cultures values not exceeding 0.2.

7

8      Although two of the interferons, Roferon A<sup>TM</sup> and  
9      Multiferon<sup>TM</sup> showed a tendency to increase baseline  
10     levels in the cytotoxicity assay, the result showed  
11     no dose-dependent increase in these levels and the  
12     OD values did not exceed 0.6 in any case. There was  
13     no similar tendency for Intron A<sup>TM</sup> or Betaferon<sup>TM</sup>.  
14     The concentration of interferons capable of  
15     decreasing OD values of virus-infected cultures by  
16     50% (IC<sub>50</sub>) are shown in Table 4 which shows the  
17     results of experiments where IFN was added either  
18     once (type 1) or twice (type 2) to the cells.

19

Interferon	IC <sub>50</sub> Exp. Type 1		IL <sub>50</sub> Exp. Type 2	
	10 TCID <sub>50</sub>	100 TCID <sub>50</sub>	10 TCID <sub>50</sub>	100 TCID <sub>50</sub>
Betaferon	110	625	110	190
Multiferon	540	2400	490	2200
Intron A	>3.125	>3.125	>3.125	>3.125
Roferon	>3.125	>3.125	>3.125	>3.125

20

21     **Table 4.** Effect of various interferons on SARS-HCoV  
22     replication

23

24     IC<sub>50</sub> values given as IU of interferon per ml. Slight  
25     inhibition of cytotoxicity was obtained with

1 Roferon™ as well as Intron A™ at the highest  
2 concentrations tested, but the reduction of OD  
3 values did not reach the 50% level in any experiment  
4 with these interferons.

5

6 The outcome of the two different experiments  
7 performed were similar, showing that Betaferon™ had  
8 the highest antiviral activity ( $IC_{50}$  50-500 IU/ml)  
9 followed by Multiferon™ ( $IC_{50}$  500-2000 IU/ml).  
10 Neither Intron A™ nor Roferon™ had any clear  
11 antiviral activity at the highest concentrations  
12 used in the experiments (3.125 IU/ml). Extrapolation  
13 of results obtained with the highest concentrations  
14 of the IFN preparations showed that  $IC_{50}$  levels could  
15 be expected to be reached at concentrations of  
16 10,000-15,000 IU/ml for the latter two types of IFN-  
17  $\alpha$ .

18

19 Discussion

20 The present results corroborate earlier findings  
21 that IFN- $\beta$  has an antiviral activity against the  
22 SARS-HCoV, that is superior to that of recombinant,  
23 IFN- $\alpha$ 2, interferons (Cinatl *et al.*, 2003).  
24 Furthermore, the results indicate that multi-  
25 subtype, natural IFN- $\alpha$ , albeit being less active  
26 that  $\beta$ -interferon, also has a significant effect on  
27 SARS-HCoV replication. The latter finding agrees  
28 with the recent results by Tan *et al.* (2004) who  
29 found, using a plaque reduction assay, that two  
30 types of natural IFN- $\alpha$  preparations showed strong

1 anti SARS-HCoV activity with a potency that was only  
2 slightly lower than that obtained with  $\beta$ -interferon.

3

4 The accumulated evidence now suggests that  
5 interferons may have a role in the treatment of  
6 severe acute respiratory syndrome (SARS)  
7 coronavirus. The promising results of Loutfy et al.  
8 (2003) were obtained using a recombinant so-called  
9 consensus IFN- $\alpha$  (Infergen) that is believed to have  
10 effects that are shared by various subtypes of IFN- $\alpha$ . The suggestive clinically beneficial effect of  
11 the consensus IFN- $\alpha$  may be concordant with the  
12 presently obtained in vitro results with nIFN- $\alpha$ , but  
13 as far as we are aware, no studies on the relative  
14 in vitro activities of nIFN- $\alpha$  and consensus IFN- $\alpha$   
15 have been performed.

16

17

18 Example 3 - Anti-viral effect of multi-subtype  
19 interferon as compared to Intron A against Semliki  
20 Forest Virus in Vero E6 cells

21

22 Vero E6 cells were seeded in 96-well plate, at a  
23 density of 10000 cells per well. After incubation  
24 overnight at 37°C, cells were incubated with 100ul  
25 of a serial 10-fold dilution of Multiferon or Intron  
26 A (titration range from 1250 IU/ml - 2.4 IU/ml).  
27 After 24 hours, cells were infected with 5000 pfu of  
28 Semliki Forest Virus (estimated MOI was 0.1) and  
29 further incubated for 48 hours until cytopathic  
30 effect was observed in untreated wells. Media was  
31 removed from cells, and cells were washed in 1 x

1 PBS, then fixed for 10 minutes at room temperature  
2 in 4% paraformaldehyde in PBS. Paraformaldehyde was  
3 removed and cells were stained with 0.2% crystal  
4 violet in 2% ethanol for 10 minutes at room  
5 temperature. Stained plates were washed and degree  
6 of colouration was quantified at 630nm using an  
7 ELISA reader. Triplicate data is presented in graph  
8 format (Figure 2).

9

10 Results

11 Figure 2 demonstrates that Multiferon was found to  
12 be effective at protecting Vero E6 cells from SFV  
13 infection over a range of concentrations. At 625  
14 IU/ml, the same degree of protection was observed  
15 for both Multiferon and IntronA (results not shown),  
16 and an equivalent loss of protection was observed  
17 for both products at 39 IU/ml. At all  
18 concentrations in between, Multiferon provided  
19 significantly higher protection than provided by  
20 Intron A.

21

22 Example 4 - Anti-viral effect of multi-subtype  
23 interferon in Human Cells

24

25 Multiferon™ was added prior to addition of the  
26 virus. The human Encephalomyocarditis virus (EMCV)  
27 was then used to infect A549 cells and the effect of  
28 Multiferon™ on the cytopathogenicity of EMCV was  
29 determined by assessing the interferon concentration  
30 required to obtain 50% cytopathic effect (CPE) for  
31 the human A549 cells. Results are shown in Figure

1       3. Cell survival was measured photometrically and  
2       results are shown in Figure 4.

3

4       The results show that the Multiferon™ preparation  
5       successfully protected against a cytopathic effect  
6       on EMCV-infected cells and that the adverse effect  
7       on the host cells did not continue to rise  
8       significantly at effective Multiferon™  
9       concentrations.

10

11       Figure 3 shows the concentrations of Multiferon™  
12       needed to obtain 50% cytopathic effect in the human  
13       cells at varying viral titres. As would be  
14       expected, a higher viral concentration requires a  
15       higher effective Multiferon™ concentration.

16

17       Figure 4 shows that Multiferon™ does not have  
18       significant adverse cell toxicity effects on human  
19       host cells.

20

21       Discussion

22       The results provided show that many interferons are  
23       highly effective at inhibiting the activity of the  
24       SARS-HCoV. Further, it has been shown that, in  
25       general natural interferons, especially multi sub-  
26       type interferons, such as Multiferon™, are  
27       particularly effective. Moreover at effective  
28       Multiferon™ concentrations, no cytotoxicity is  
29       observed.

30

31       In tests for anti-viral activity in human cells,  
32       Multiferon™ shows a good dose response with

1       cytotoxicity levels which do not rise in proportion  
2       to the effective Multiferon™ concentration.

3

4       These results indicate that certain interferons such  
5       as Multiferon™ are highly effective therapeutics for  
6       the treatment of SARS-HCoV infection in humans and  
7       can be expected to have low levels of adverse  
8       effects *in vivo*.

9

10      Other groups have studied the efficacy of  
11      recombinant interferon products against SARS CoV.  
12      Stoher et al demonstrated significant but incomplete  
13      activity of Intron A at a concentration of 1000-5000  
14      IU/ml on cells infected with a multiplicity of  
15      infection (MOI) of 0.001 plaque forming units per  
16      cell in a cytopathic endpoint assay. However, the  
17      results presented show that Multiferon™ used at the  
18      low dose of 5 IU/ml completely protected cells from  
19      SARS-HCoV infection at a MOI of 0.005 plaque forming  
20      units per cell, five times greater than the MOI used  
21      in the Intron A™ experiments. Furthermore, 50 IU/ml  
22      of Multiferon™ protected cells from SARS-HCoV  
23      infection at a MOI of 0.05, 50 times greater than  
24      the MOI utilised in the Intron A™ studies. Finally,  
25      in our studies, concentrations of Intron A™ or  
26      Roferon™ up to 100000 and 500000 IU/ml,  
27      respectively, failed to fully protect cells from  
28      SARS-HCoV infection.

29

30      Whilst Stoher et al. claim that doses of up to 3.6 x  
31      10<sup>7</sup> IU/ml have been infused intravenously, and that  
32      serum concentrations of at least 500 IU/ml are

1 achievable after intramuscular injection, the serum  
2 titre would only reach this level for a short period  
3 of time, and intravenous infusion has highly toxic  
4 implications. Taken together with the results  
5 described, this supports the significant superiority  
6 of natural multi-subtype interferon products, in  
7 particular Multiferon™, over recombinant IFN alpha2  
8 preparations.

9

10 All publications and patent documents referred to  
11 herein are incorporated by reference in their  
12 entirety. Although the invention has been described  
13 in connection with specific examples, it should be  
14 understood that the invention should not be unduly  
15 limited to such examples. Specifically, it will be  
16 understood by one skilled in the art that various  
17 modifications to and variations of the invention as  
18 described herein may be made without departing from  
19 the scope of the invention.

20

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1       **Claims**

2

3       1. A method of treating or preventing a  
4            coronaviral infection, the method including the  
5            step of administering a therapeutically useful  
6            amount of an interferon to a subject in need of  
7            treatment.

8

9       2. A method as claimed in claim 1, wherein the  
10            interferon is interferon alpha or interferon  
11            beta.

12

13       3. A method as claimed in claim 1 or claim 2  
14            wherein the interferon is selected from the  
15            group consisting of multi-subtype interferon  
16            alpha (IFN $\alpha$ ), interferon  $\alpha$ n1, interferon  $\alpha$ n3 or  
17            interferon  $\beta$ 1b.

18

19       4. A method as claimed in any one of claims 1 to 3  
20            wherein the interferon is derived from human  
21            cells.

22

23       5. A method as claimed in any one of claims 1 to 3  
24            wherein the interferon is recombinant.

25

26       6. A method as claimed in any one of claims 1 to  
27            5 wherein the interferon is an isolated  
28            interferon.

29

30       7. A method as claimed in any preceding claim  
31            wherein the interferon is multi-subtype, human

1       alpha-interferon derived from white blood cells  
2       commercially available as Multiferon™.

3

4       8. A method as claimed in any preceding claim  
5       wherein the coronavirus infection is a human  
6       coronaviral infection.

7

8       9. A method as claimed in any preceding claim  
9       wherein the coronaviral infection is severe  
10       acute respiratory syndrome (SARS) coronavirus  
11       (SARS-HCoV).

12

13       10.      Use of interferon in the treatment of a  
14       human coronaviral infection.

15

16       11.      Use of interferon in the prevention of a  
17       human coronaviral infection.

18

19       12.      Use of interferon as claimed in claims 10  
20       or 11 wherein the interferon is interferon  
21       alpha or interferon beta.

22

23       13.      Use of interferon as claimed in claims 10  
24       or 11 wherein the interferon is multi-subtype  
25       interferon alpha (IFN $\alpha$ ), interferon  $\alpha$ n1,  
26       interferon  $\alpha$ n3 or interferon  $\beta$ 1b.

27

28       14.      Use of interferon as claimed in any one of  
29       claims 10 to 13 wherein the interferon is the  
30       multi-subtype, human alpha-interferon derived  
31       from white blood cells commercially available  
32       as Multiferon™.

1

2       15.     Use of interferon as claimed in any one of  
3            claims 10 to 13 wherein the interferon is  
4            recombinant.

5

6       16.     Use of interferon as claimed in any one of  
7            claims 10 to 15 wherein the coronaviral  
8            infection is a human coronavirus.

9

10      17.     Use of interferon as claimed in any one of  
11            claims 10 to 16 wherein the coronaviral  
12            infection is severe acute respiratory syndrome  
13            (SARS) coronavirus (SARS-HCoV).

14

15      18.     A method of treating human infection with  
16            a coronavirus, the method including the step of  
17            administering a therapeutically useful amount  
18            of an interferon to a subject in need of  
19            treatment along with a therapeutically useful  
20            amount of a suitable anti-viral compound.

21

22      19.     A method as claimed in claim 18 wherein  
23            the coronavirus is severe acute respiratory  
24            system (SARS) coronavirus (SARS-HCoV).

25

26      20.     A method as claimed in claims 18 or 19  
27            wherein the anti-viral compound is ribavirin.

28

29      21.     Use of interferon and an anti-viral  
30            compound in the preparation of a combined  
31            medicament for the treatment or prevention of  
32            infection with a coronavirus.

1

2 22. Use of interferon and an anti-viral  
3 compound as claimed in claim 21 wherein the  
4 coronavirus infection is severe acute  
5 respiratory system (SARS) coronavirus (SARS-  
6 HCoV).

7

8 23. An assay method for determining the  
9 efficacy of a candidate agent in the treatment  
10 of a coronaviral infection, the assay method  
11 including the steps of;

12 - incubating cells infected with coronavirus  
13 in the presence of the candidate agent,  
14 - determining the degree of inhibition of  
15 the cytopathic effect of the virus on the  
16 cells, and  
17 - comparing the degree of inhibition  
18 obtained using the candidate agent with  
19 the degree of inhibition obtainable with  
20 incubation with an interferon or  
21 interferon based product.

22

23 24. An assay as claimed in claim 23 wherein  
24 the interferon is a multi-subtype interferon.

25

26 25. An assay as claimed in claim 24 wherein  
27 the multi-subtype interferon is Multiferon™.

28

29 26. An assay method for determining the  
30 efficacy of a candidate agent in the prevention  
31 of a coronaviral infection, wherein the assay  
32 method includes the steps of:

1        -incubating cells in the presence of the candidate  
2        agent,  
3        -adding the coronavirus to the cells, and  
4        -determining the degree of protection against the  
5        coronaviral infection afforded by the candidate  
6        agent.

7

8        27.       An assay as claimed in claim 26 wherein  
9               the interferon is a multi-subtype interferon.

10

11       28.       An assay as claimed in claims 26 or 27  
12               wherein the multi-subtype interferon is  
13               Multiferon™.

14

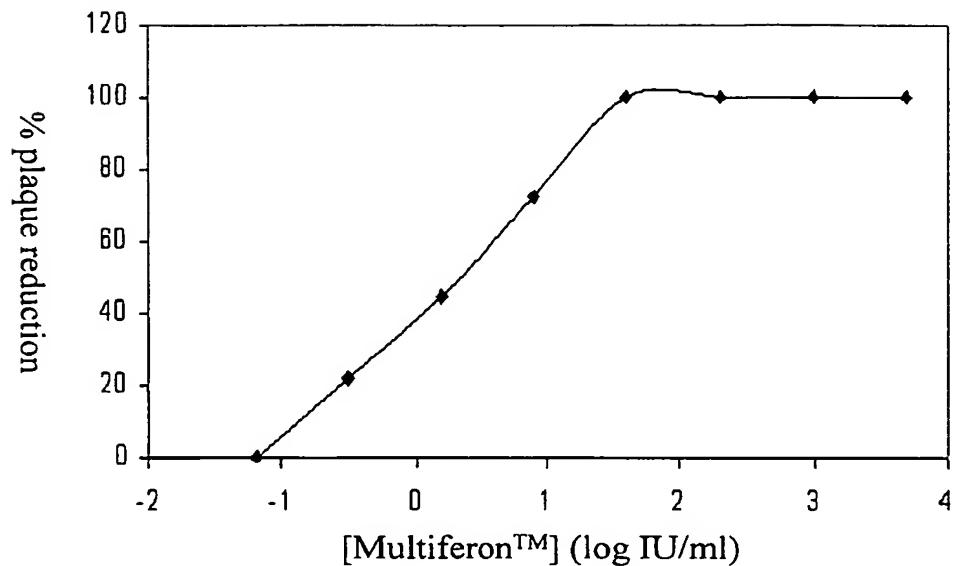
15       29.       Use of interferon in the manufacture of a  
16               medicament for the treatment of a human  
17               coronavirus.

18

19       30.       Use of interferon as claimed in claim 29  
20               wherein the interferon is multi-subtype, human  
21               alpha-interferon derived from white blood cells  
22               commercially available as Multiferon™.

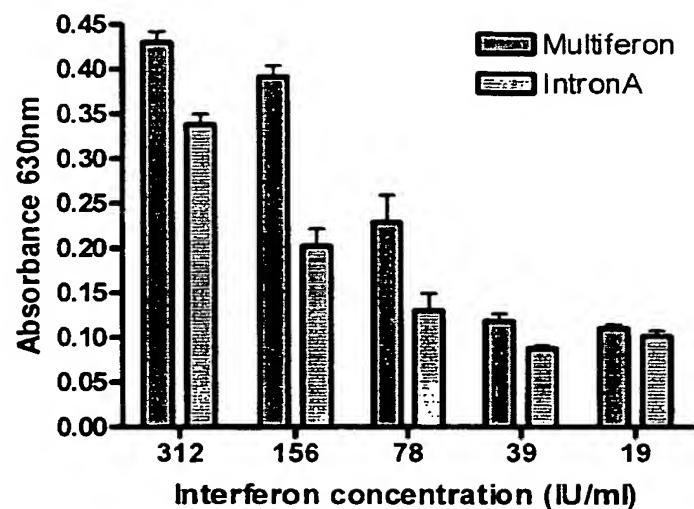
23

1/4



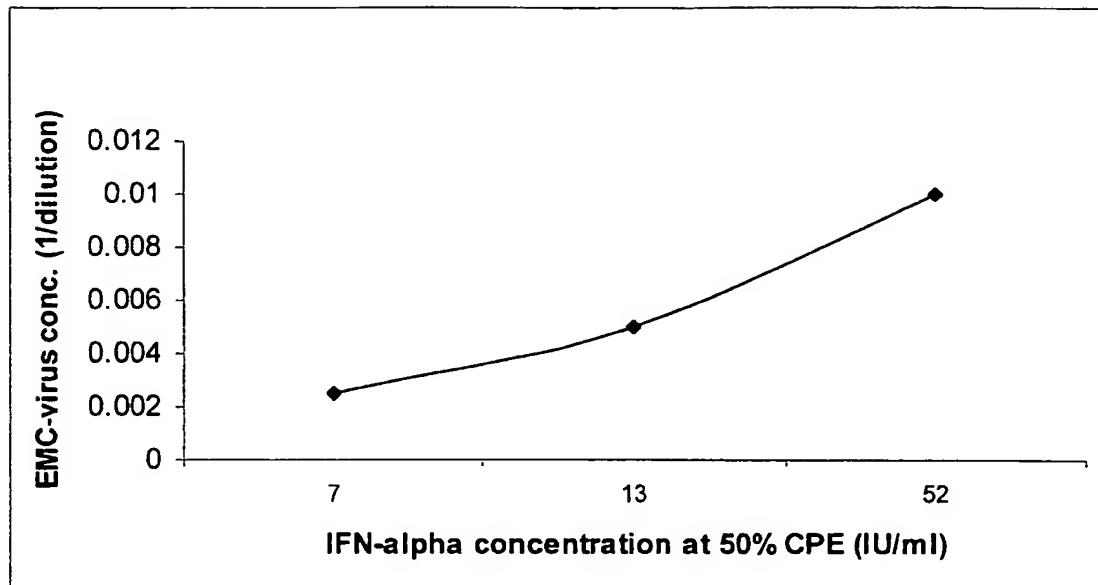
**Figure 1: Effect of Multiferon™ against the SARS-HCoV by plaque reduction (Virus concentration is 54 pfu/ml (IU = International Units))**

2/4



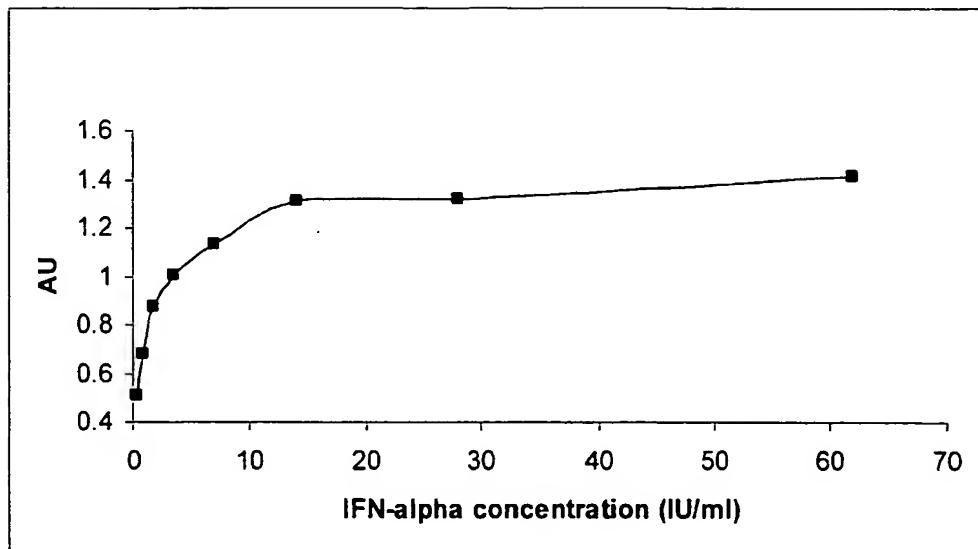
**Figure 2: Cytopathic endpoint assay in Vero E6 cells infected with Semliki Forest virus, and treated with a serial titration of Multiferon or Intron A**

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**Figure 3: Effect of Multiferon on cytopathogenicity of EMCV on A549 cells.** (The Multiferon concentration required to obtain 50 % cytopathic effect (CPE) for human A549 cells challenged with EMC-virus is shown for different concentrations of EMC-virus, presented as 1/dilution).

4/4



**Figure 4: Effect of increasing concentrations of Multiferon™ on survival of A549 cells.** Cell survival, measured photometrically at  $\text{Abs}_{595\text{nm}}$ , using a fixed dilution of EMCV (dilution 1/400), at increasing concentrations of Multiferon. (AU = Absorbance Units)

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/GB2004/002183

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 A61K38/21 A61K31/7056 A61P31/14

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, EMBASE, PASCAL

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	CINATL J ET AL: "Treatment of SARS with human interferons" LANCET THE, LANCET LIMITED. LONDON, GB, vol. 362, no. 9380, 26 July 2003 (2003-07-26), pages 293-294, XP004441882 ISSN: 0140-6736 the whole document ----- DATABASE MEDLINE 'Online' US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US; June 2003 (2003-06), GAO ZHAN-CHENG ET AL: "Clinical investigation of outbreak of nosocomial severe acute respiratory syndrome!" XP002294985 Database accession no. NLM12837162 abstract	1-6, 8-13, 15-17, 23,26,29
P, X	----- -/-	1,2,5,6, 8-12, 15-22,29

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

## \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*Z\* document member of the same patent family

Date of the actual completion of the international search

6 September 2004

Date of mailing of the international search report

21/09/2004

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB2004/002183

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	<p>&amp; ZHONGGUO WEI ZHONG BING JI JIU YI XUE = CHINESE CRITICAL CARE MEDICINE = ZHONGGUO WEIZHONGBING JIJIUYIXUE. JUN 2003, vol. 15, no. 6, June 2003 (2003-06), pages 332-335, ISSN: 1003-0603</p> <p>LAU ARTHUR CHUN-WING ET AL: "Severe acute respiratory syndrome treatment: present status and future strategy." CURRENT OPINION IN INVESTIGATIONAL DRUGS (LONDON, ENGLAND : 2000) AUG 2003, vol. 4, no. 8, August 2003 (2003-08), pages 918-920, XP009035955 ISSN: 1472-4472 page 919, right-hand column, paragraph 4</p> <p>ZHAO Z ET AL: "Description and clinical treatment of an early outbreak of severe acute respiratory syndrome (SARS) in Guangzhou, PR China" JOURNAL OF MEDICAL MICROBIOLOGY 01 AUG 2003 UNITED KINGDOM, vol. 52, no. 8, 1 August 2003 (2003-08-01), pages 715-720, XP009035954 ISSN: 0022-2615 treatment gropus B, C, D page 717</p> <p>HIGGINS P G ET AL: "INTRANASAL INTERFERON AS PROTECTION AGAINST EXPERIMENTAL RESPIRATORY CORONAVIRUS INFECTION IN VOLUNTEERS" ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON, DC, US, vol. 24, no. 5, November 1983 (1983-11), pages 713-715, XP009016436 ISSN: 0066-4804 abstract</p> <p>HUSA P: "What Is the Role of Leucocyte Interferon Alfa in the Treatment of Chronic Hepatitis C in the Time of Pegylated Interferons?" CESKA A SLOVENSKA GASTROENTEROLOGIE A HEPATOLOGIE 2003 CZECH REPUBLIC, vol. 57, no. 5, 2003, pages 189-193, XP009035953 ISSN: 1213-323X abstract</p>	1,2,6, 8-12, 16-20, 22,29
P, X		1,2,5,6, 8-12, 15-19, 21,22,29
X		1,2,4-6, 8,10-12, 15,16,29
Y		3,7,13, 14,30
Y		3,7,13, 14,30
		-/--

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/GB2004/002183

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 1986, TURNER R B ET AL: "PREVENTION OF EXPERIMENTAL CORONAVIRUS COLDS WITH INTRANASAL ALPHA-2B INTERFERON" XP002294986 Database accession no. PREV198682100287 abstract &amp; JOURNAL OF INFECTIOUS DISEASES, vol. 154, no. 3, 1986, pages 443-447, ISSN: 0022-1899</p> <p>-----</p> <p>DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; August 2000 (2000-08), MATSUYAMA S ET AL: "Protective effects of murine recombinant interferon-beta administered by intravenous, intramuscular or subcutaneous route on mouse hepatitis virus infection" XP002294987 Database accession no. PREV200000499341 abstract &amp; ANTIVIRAL RESEARCH, vol. 47, no. 2, August 2000 (2000-08), pages 131-137, ISSN: 0166-3542</p> <p>-----</p> <p>MYINT S H: "Human coronaviruses: A brief review" REVIEWS IN MEDICAL VIROLOGY 1994 UNITED KINGDOM, vol. 4, no. 1, 1994, pages 35-46, XP009035949 ISSN: 1052-9276 page 43, left-hand column, paragraph 5</p> <p>-----</p> <p>PEIRIS J S M ET AL: "Coronavirus as a possible cause of severe acute respiratory syndrome." LANCET (NORTH AMERICAN EDITION), vol. 361, no. 9366, 19 April 2003 (2003-04-19), pages 1319-1325, XP002295043 ISSN: 0099-5355 abstract</p> <p>-----</p>	1,2,5,6, 8,10-12, 15,16,29
X		1,2,5,6
X		1,2,8, 10-12, 16,29
A		1-30

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/GB2004/002183

### Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:  
Claims 1-20: Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy
2.  Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

#### Remark on Protest

The additional search fees were accompanied by the applicant's protest.  
 No protest accompanied the payment of additional search fees.